Opportunities for confinement of rice

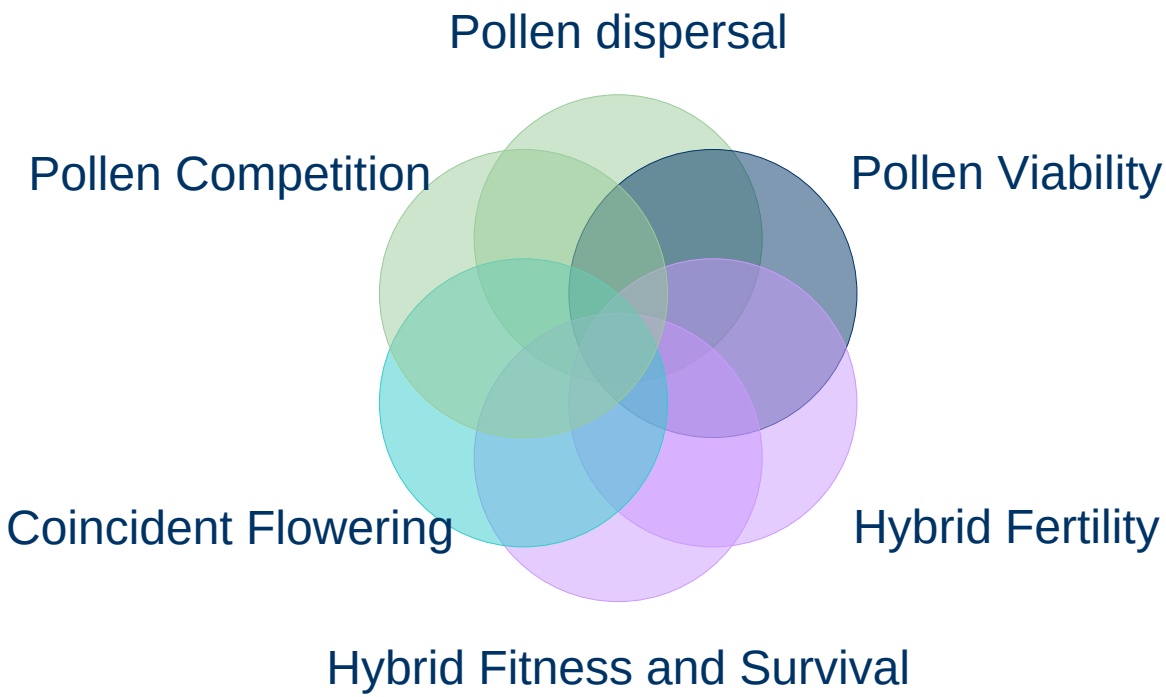
USDA Workshop on Confinement of Genetically Engineered Crops During Field Testing

Presented by Donna Mitten
September 14, 2004
Greenbelt, MD USA

Why Rice?

- The biology of cultivated rice provides many safeguards for confinement.
- Current rice production services specific markets based upon grain type and quality.
- Market segmentation is facilitated by regional production and grain handling.
- Thus, rice an ideal crop for production of high value proteins in the USA

Biological barriers to gene flow in rice

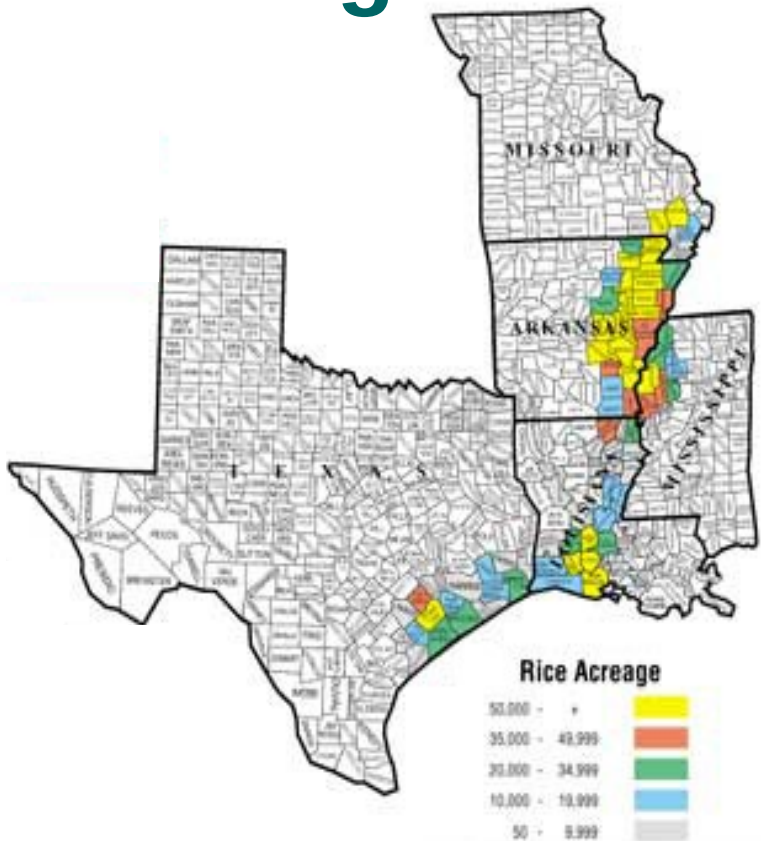


Production system safeguards

- Control of planting seed
- Opportunity for distinct production and markets for different types of rice
  - Commodity and specialty food rice systems
  - Separate and closed system for protein rice
- Commercial scale
  - Food rice in USA is 2.5 million acre
  - Protein rice - estimated max at 40,000 acres

Six distinct production regions

- the Arkansas Grand Prairie
- northeastern Arkansas and the bootheel of Missouri
- the Mississippi River Delta (in Arkansas, Mississippi, and northeast Louisiana)
- southwest Louisiana
- the Coastal Prairie of Texas
- California's Sacramento Valley



- Rice storage and mills are located within each production region.

Source of Map: Rice Journal, 2003 season

Biological safeguards

- Rice is self-pollinated
- Temperate rice survives only in a highly managed agro-eco system
- Pollen dispersal baseline studies conducted in US with glufosinate-tolerant rice confirm safeguards.
- If time permits, we can review the pollen dispersal studies in LA and CA.

Rice pollen dispersal

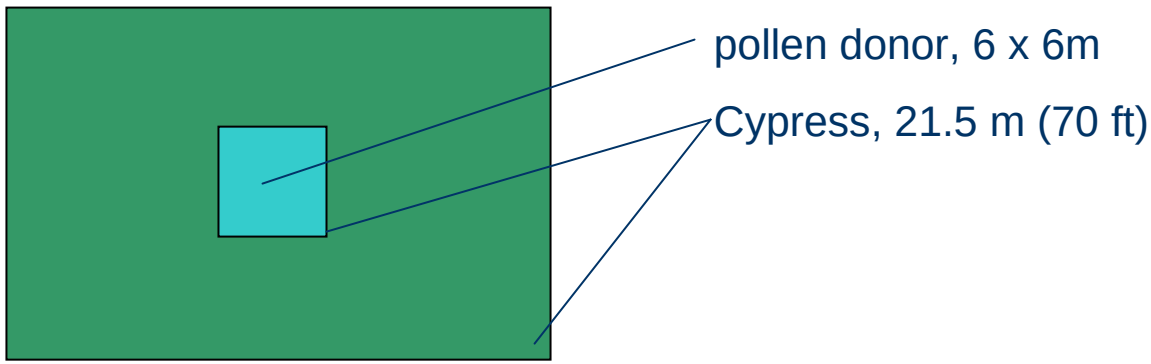
- Joint work by Pinson (USDA) and Linscombe (LSU)
- LSU Rice Research Station, Crowley, Louisiana and USDA Rice Station in Beaumont, Texas - coincident flowering. LLRICE62 was the pollen donor.
- 6 confirmed hybrids in 7,700 seed or 0.08% outcrossing was measured where the transgenic and non-transgenic rice plants were inter-seeded.
- No survivors were identified elsewhere in the study.
- The study confirmed the certified seed isolation standard of 4.6m (15ft) as appropriate for herbicide-tolerant rice.

Field phase completed by
Steve Linscombe and Xueyan Sha of the LSU AgCenter

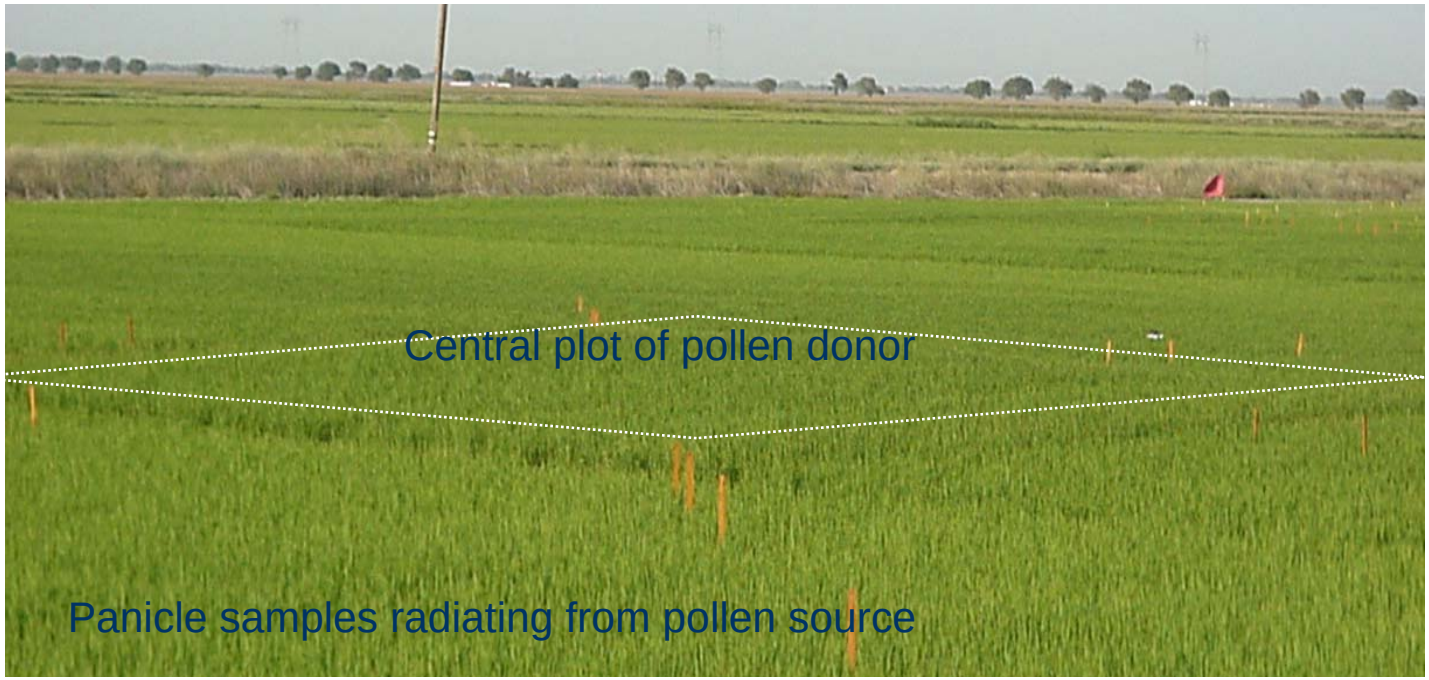
LSU pollen dispersal study

- The field design used a central plot 6m^2 seeded with 90% LLRICE62 (medium grain variety, LL401) and 10% pollen receptor, the long grain variety Cypress.
- The central plot was surrounded by Cypress.
- Flowering was optimal for outcrossing.
- At maturity, 50 panicles sampled at border of central plot and 2.3, 4.6, 9, 13.8, 21.5 m in 8 directions.

Certified seed isolation distance is 4.6 m (15 ft)



Design of LA study



Lab phase completed by Shannon Pinson, Faye Seaberg of the USDA

Screening for survivors

- 820 intact panicles (74,689 seed) tested for germination in 0.1% solution of commercial Liberty® herbicide (20% glufosinate) by lab bioassay developed by Pinson and Seaberg. Tested were Cypress from the center plot, borders, 2.3, 4.6 and 21.5 m.
- No clusters of pollination were observed in the panicle tests.
- In addition, 80,000 bulk seed were tested in lab bioassay from the extreme (21.5m) and the border samples.
- In all >120,000 germinating seeds were screened.
- 20 survivors, all from the center plot, were established in the greenhouse for further testing.

Photos from Faye Seaberg of the USDA Rice Station in Beaumont

Screening for survivors

- Lab bioassays
  - labor intensive
  - dependent upon consistent conditions and technical experience to read the test
  - limited numbers can be screened (huge effort to do 100,000 seed)
  - avoid field release

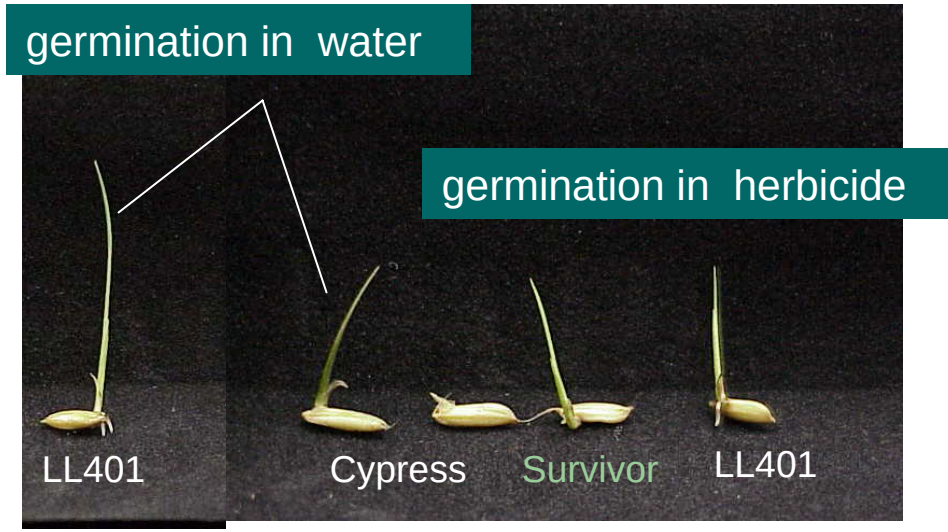


Photo from
Albert Fischer
UC-Davis

Screening for survivors

- Field screening
  - massive (1million seed possible) but space required to conduct the test
  - clear identification
  - germination never 100%

Survivor following Liberty® herbicide application

PCR by Wuzi Xie,
Bayer CropScience

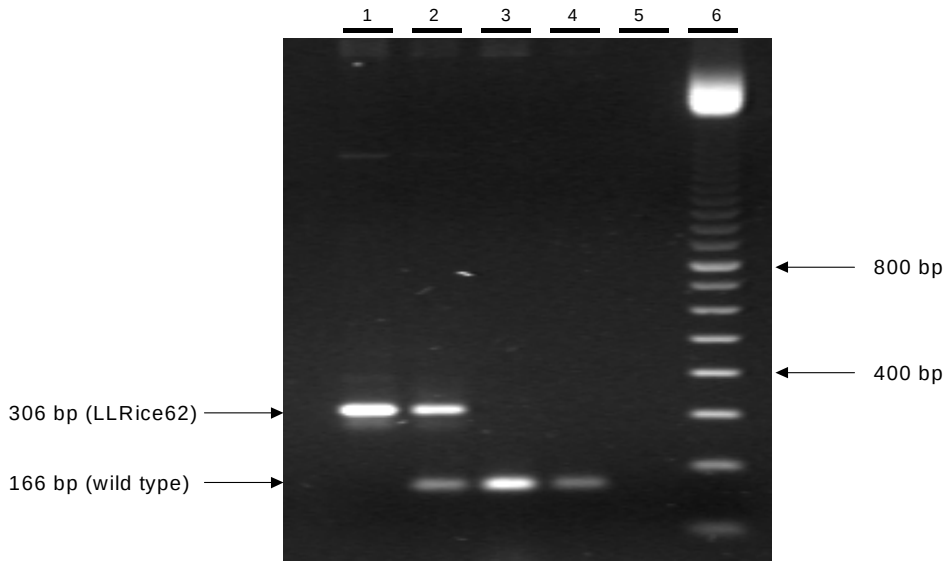
Confirmation of hybrids

- Leaf samples of the 20 survivors in the bioassay were tested using a PCR protocol designed to discriminate between plants either homozygous, wild type and hemizygous (hybrid) for the LLRICE62 genetic locus.
  - 6 were confirmed to be the result of outcrossing
  - 1 was confirmed to be LLRICE62, and
  - the rest were Cypress plants.

- 6 confirmed hybrids in 7,700 seed or 0.07%
- Hybrids found only in the interplanted plot

PCR by Wuzi Xie,
Bayer CropScience

LLRICE62 Zygosity Scoring PCR



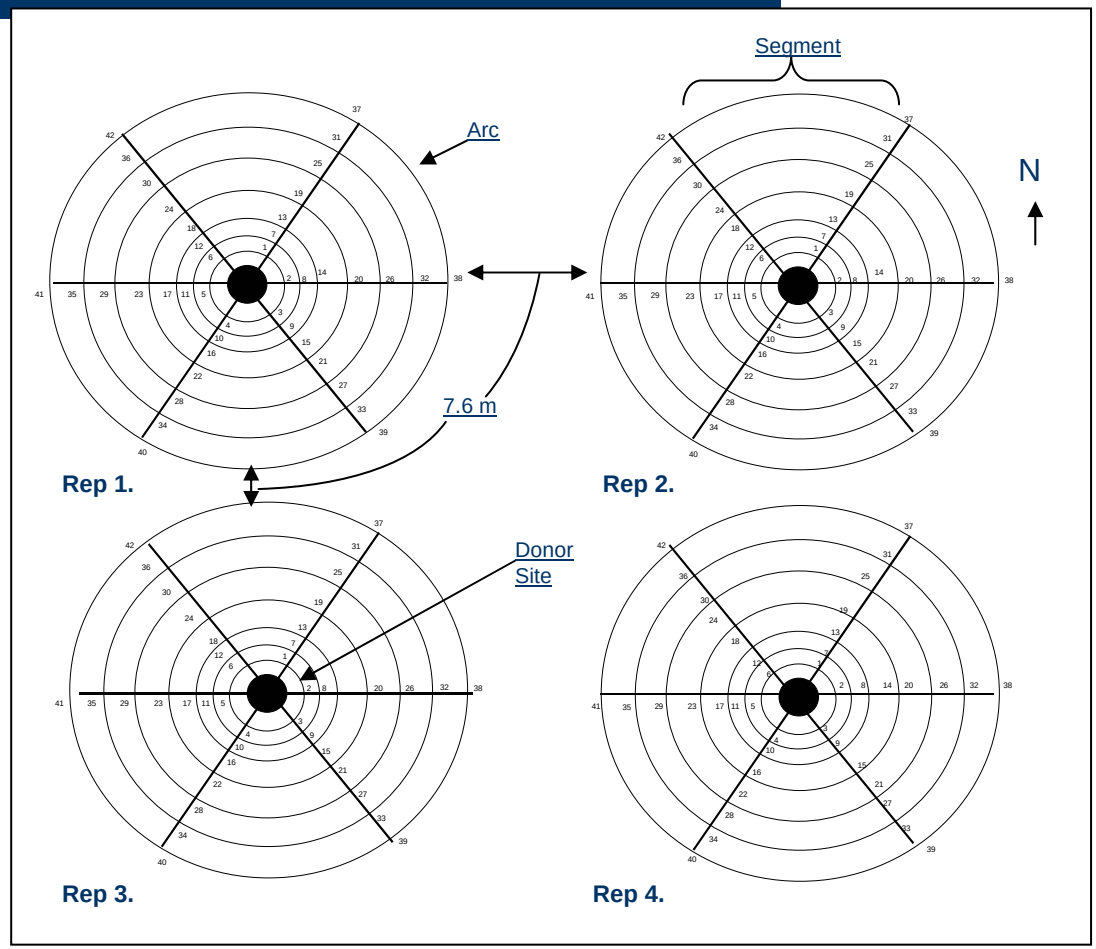
OSR-LL62-ZY01

Lane 1: Homozygous LLRice62. Lane 2: Hemizygous LLRice62. Lane 3: *O.sativa* var. Bengal, wild type. Lane 4: *O.sativa* var. M202, wild type. Lane 5: water (No Template Control). Lane 6: MW marker (Pharmacia 100 bp ladder)

David Cheetham and
Albert Fischer, UCD

Design of California study

- two seasons
- four blocks
- 4.6 m donor
- 16.9 m receptor
- n = 10,000 seed
- high confidence
- allow detection of 0.001%



Summary of California study

- no outcrossing detected beyond 1.9 m
- range detected 0.01 to 0.4%
- in excess of 1.8 million seed screened (188 samples x 10,000 seed)
- recommendations for isolation, the distance of a farm road, 6.2 m, between fields